INNOVATIVE MINIMALLY-INVASIVE ANALYTICAL STRATEGIES FOR HUMAN BIOMONITORING: MEASUREMENT OF TOXICANTS IN DRIED-BLOOD SPOTS

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Introduction

The concept of sampling newborn infants for a few microliters of blood to screen for inherited endocrine, nutritional, or metabolic disorders has been introduced by Guthrie at the University of Buffalo in 1963[1]. Newborn screening for additional diseases has since been extensively performed in North America and Europe (Newborn Screening Programs - NSPs), but also in developing countries because of the ease of collection, transport, and storage, as well as the reduced risk of contamination of the handlers due to infectious pathogens, compared to the use of classical liquid specimens. Human dried-blood spots (DBS) are generally simply obtained by pricking the heel or finger by using single-use lancing devices to sample a few microliters (50-150 µl) of capillary blood. The blood is then collected on a piece of filter paper made of high purity cotton linters[2]. After drying, DBS are stored in plastic bags at ambient temperature. For analysis, part of the spot is punched out (6 mm punches) and the blood is eluted using various aqueous solutions[3]. In recent years, DBS testing further evolved towards more extensive testing due to the availability of more sensitive and specific methodologies. Next to screening for congenital diseases and viruses, DBS from NSPs have thus lately also been considered for exposure to toxicant assessment. To the best of our knowledge, only Dua *et al.* and Burse *et al.* briefly reported preliminary data on the potential use of DBS for hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE) measurement using GC coupled to non-selective micro-electron capture detector (µECD)[4,5].

The aim of the work is to develop new analytical strategies to measure selected representative POPs (or metabolites or reaction products) in DBS to assess internal dose exposure by means of innovative minimally-invasive biomonitoring. The methodology is be based on cryogenic modulation of gas chromatographic signals, either applied to comprehensive two-dimensional gas chromatography (GCxGC), or to cryogenic zone compression (CZC)-GC. Both GC approaches to be hyphenated to high resolution (HR) mass spectrometric (MS) analyzers.

Materials and Methods

Chemicals

All chemicals and instrumentation are similar to those used for dioxin routine analyses under ISO17025 QA/QC requirements. A human serum QC pool was used for optimization and testing. This pool is naturally contaminated and is representative of the general European population background levels. The target analytes consisted in DDE, PCB-153. They can be considered as indicator of PCB and organochlorine pesticide (OCP) levels.

Analytical procedure

For method development, sample sizes ranged between 200 µL and 1 mL. Each sample was liquid-liquid extracted using hexane. Briefly, samples were placed in 10 mL conical bottom polypropylene Falcon™ tubes. The extraction was carried out with three times 5 mL of hexane and wrist-action shaking. The solvent extract was evaporated in a PowerVap 6 system (Fluid Management Systems Inc., Waltham, MA, USA) to 500 µL using regular evaporation tubes. This volume was loaded on a multilayer micro column made of 0.4g of sodium sulphate and 0.5g of 22% sulphuric acid silicagel, from top to bottom. The eluate (3 x 2 mL of hexane) was concentrated to 400 µL in the PowerVap using GC-vial connected evaporation tubes. Final solvent reduction took place overnight at room temperature after 5 µL of nonane was added as keeper. The final extract volume was 5 µL.

Measurements were carried out on a JEOL AccuTOF GC system (JEOL Ltd., Tokyo, Japan). The GC oven (Agilent 6890) was equipped with a ZX1 - LN_2 Cooled Loop Modulation GC x GC System (Zoex Corp., Houston, TX, USA). The ^{1}D GC column was an HT-8 (60 m x 0.25 mm ID x 0.25 µm df) (SGE, Villebon, France). The 2D GC column was an Rxi-17 (1.5 m x 0.25 mm ID x 0.25 µm df) (Restek, , Belletonte, PA, USA). The PM was 4 s, 400 ms of hot pulse duration. The temperature program was 140°C for 2 min, 15°C/min to 220°C for 7,5 min, 6°C/min to 250°C, 2°C/min to 265°C, 30°C/min to 310°C for 20 min. 1 µL of the final extract in nonane (5 µL) were injected into a split/splitless injector held at 275°C in splitless mode. Helium was used at 0.8 mL/min. The major MS parameters were an ion source temperature of 140°C, an ionisation voltage of 200 V, methane at 1 mL/min as reagent gas, an acquisition range from 30.00 to 700.00 m/z, a recording interval of 0.04 s (25 Hz), an accumulation time of 0.037 s, a data sampling interval of 0.5 ns, and a detector voltage of 2300 V. The mass accuracy of the instrument was ensured by frequent single point calibration checks.

Results and Discussion

Cryogenic zone compression (CZC)

CZC was performed to enhance instrumental LODs. The iLODs for PCB-153 were 50 fg/µL, and iLODs for DDE were 5 pg/ µL. We tried to incorporate BDE-47 in the set of analytes but the iLODs were not good enough. We used a 60m carborane GC phase to ensure proper separation of PCBs and avoid co-elution of targets with other isobaric species. The width at half height was 150 ms. This corresponded to several slices per peak, excepted for the lowest points of the calibration curve where only one slice was often observed. The calibration curve working zone ranged from 50 fg to 100 pg for PCB-153 and from 1 pg to 500 pg for DDE. Curves are illustrated in Figure 1. PCB-153 ^{13}C-labeleld standard was used to quantify both PCB-153 and DDE because ^{13}C DDE was not available at the time of experiments. This is the reason for the surprising response factor for DDE curve.

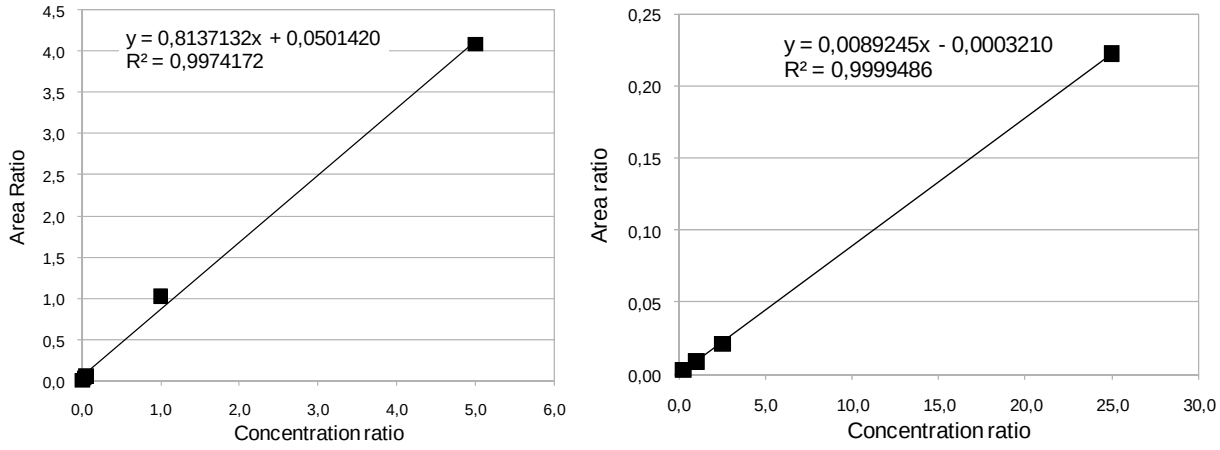

Figure 1: Calibration curves for PCB-153 and DDE.

Analyses of real human serum samples

Quality control human serum samples (500 µL) were analyzed to estimate the efficiency of the procedure. Triplicate analyses gave a mean level of 0.85 ± 0.26 pg/g lipids (29% RSD) although the reference value for that QC pool was 0.90 pg/g lipids (-4.6% relative error) for PCB-153.

Real human serum samples were also analyzed. They consisted in 500 µL specimen volumes. The internal standard was added prior extraction. Figure 2 shows the actual chromatograms for both PCB-153 and DDE naturally present in the sample. Both were quantified.

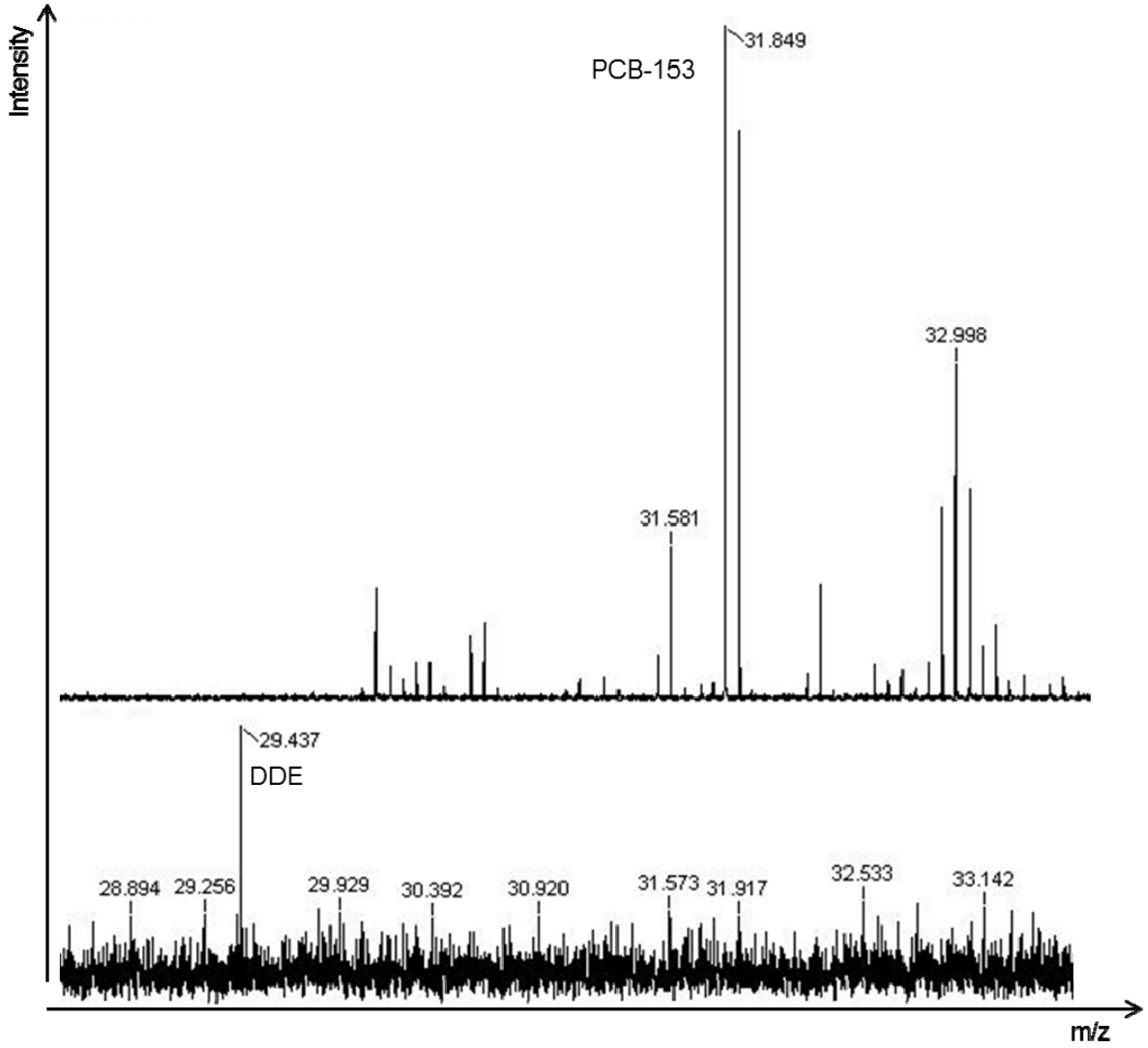

Figure 2: Signals for native PCB-153 and DDE in 500 µL of human blood analyzed by GCxGC-IDHRTOFMS.

Relative errors were around 20% or higher, especially for DDE for which measurements were performed at levels very close to iLODs. Investigation of the relatively poor accuracy revealed serious variations in the sample

preparation itself, in terms of recovery rates. Optimisation of the entire sample workflow is under consideration. No doubt that the use of ^{13}C DDE should improve the performance of the method. In terms of blank levels, no issues were encountered for DDE, but blank levels for PCB-153 were of concern and efforts should be considered to ensure proper control of contamination.

Conclusions

Those preliminary results demonstrate the feasibility of such a DBS approach for measuring selected POPs in human samples. The work performed on liquid serum samples of 500 µL has to be extended to actual whole blood BDS to further investigate the efficiency of the method. HR sector instrument also have to be used to take advantage of the sensitivity enhancement while working in selected ion monitoring (SIM) mode. Research is currently taking place in that field (see OHC this year).

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